

WHAT IS CLAIMED IS:

1 1. A protein kinase which is a member of a superfamily, said protein kinase  
2 being characterized by:  
3 A. greater than 40% sequence similarity with eEF-2 kinase from  
4 any organism; and,  
5 B. phosphorylates an amino acid within an alpha helical domain  
6 of its target protein.

1 2. A protein kinase of Claim 1 which phosphorylates eukaryotic elongation  
2 factor-2 (eEF-2), and is designated as eukaryotic elongation factor-2 kinase (eEF-2  
3 kinase).

1 3. A protein kinase of Claim 1 which phosphorylates eukaryotic myosin heavy  
2 chain (MHC), and is designated as myosin heavy chain kinase (MHCK).

1 4. A protein kinase of Claim 1 that phosphorylates a peptide sequence derived  
2 from the phosphorylation site of a target protein.

1 5. A peptide sequence having SEQ ID NO: 20.

1 6. A protein kinase of Claim 1 which is a polypeptide having an amino acid  
2 sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and  
3 SEQ ID NO: 10, and fragments thereof.

1 7. A protein kinase of Claim 1 which is derived from mammalian cells.

1 8. A protein kinase of Claim 1 labeled with a detectable label.

1 9. A protein kinase of Claim 8 wherein the label is selected from enzymes,  
2 chemicals which fluoresce, and radioactive elements.

1 10. An antibody to the protein kinase of Claim 1.

1 11. An antibody to the phosphorylated form of the target protein of Claim 1.

12. An antibody to the phosphorylated form of the peptide of Claim 5.

1 13. The antibody of Claim 10, 11 or 12 which is a polyclonal antibody.

1 14. The antibody of Claim 10, 11 or 12 which is a monoclonal antibody.

1 15. An immortal cell line that produces a monoclonal antibody according to  
2 Claim 14.

1 16. The antibody of Claim 10, 11 or 12 labeled with a detectable label.

1 17. The antibody of Claim 10, 11 or 12 wherein the label is selected from  
2 enzymes, chemicals which fluoresce and radioactive elements.

1 18. A DNA sequence which encodes eEF-2 kinase, or a fragment thereof,  
2 selected from the group consisting of:

3 (A) the DNA sequences of FIGURE 5 (SEQ ID NO: 1);

4 (B) the DNA sequences of FIGURE 5 (SEQ ID NO: 3);

5 (C) the DNA sequences of FIGURE 5 (SEQ ID NO: 9);

6 (D) DNA sequences that hybridize to any of the foregoing DNA  
7 sequences under standard hybridization conditions;

8 (E) DNA sequences that code for expression of an amino acid sequence  
9 encoded by any of the foregoing DNA sequences.

- 10 (F) degenerate variants thereof;
- 11 (G) alleles thereof; and,
- 12 (H) hybridizable fragments thereof.

1 19. A recombinant DNA molecule comprising a DNA sequence which encodes  
2 eEF-2 kinase, or a fragment thereof, selected from the group consisting of:

- 3 (A) the DNA sequences of FIGURE 5 (SEQ ID NO: 1);
- 4 (B) the DNA sequences of FIGURE 5 (SEQ ID NO: 3);
- 5 (C) the DNA sequences of FIGURE 5 (SEQ ID NO: 9);
- 6 (D) DNA sequences that hybridize to any of the foregoing DNA  
7 sequences under standard hybridization conditions;
- 8 (E) DNA sequences that code for expression of an amino acid sequence  
9 encoded by any of the foregoing DNA sequences.
- 10 (F) degenerate variants thereof;
- 11 (G) alleles thereof; and,
- 12 (H) hybridizable fragments thereof.

1 20. The recombinant DNA molecule of either of Claims 18 or 19, wherein said  
2 DNA sequence is operatively linked to an expression control sequence.

1 21. The recombinant DNA molecule of Claim 20, wherein said expression  
2 control sequence is selected from the group consisting of the early or late promoters  
3 of SV40 or adenovirus, the *lac* system, the *trp* system, the *TAC* system, the *TRC*  
4 system, the major operator and promoter regions of phage  $\lambda$ , the control regions of  
5 fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid  
6 phosphatase and the promoters of the yeast  $\alpha$ -mating factors.

1 22. A probe capable of screening for eEF-2 kinase in alternate species prepared  
2 from the DNA sequence of Claim 18.

1 23. A probe capable of screening for members of the protein kinase superfamily  
2 of Claim 1 prepared from the DNA sequence of Claim 18.

1 24. A unicellular host transformed with a recombinant DNA molecule  
2 comprising a DNA sequence or degenerate variant thereof, which encodes a protein  
3 kinase, or a fragment thereof, selected from the group consisting of:  
4 (A) the DNA sequences of FIGURE 5 (SEQ ID NO: 1);  
5 (B) the DNA sequences of FIGURE 5 (SEQ ID NO: 3);  
6 (C) the DNA sequences of FIGURE 5 (SEQ ID NO: 9);  
7 (D) DNA sequences that hybridize to any of the foregoing DNA  
8 sequences under standard hybridization conditions; and  
9 (E) DNA sequences that code on expression for an amino acid sequence  
10 encoded by any of the foregoing DNA sequences;  
11 wherein said DNA sequence is operatively linked to an expression control  
12 sequence.

1 25. The unicellular host of Claim 24 wherein the unicellular host is selected  
2 from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeasts,  
3 CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells, plant  
4 cells, insect cells, and human cells in tissue culture.

1 26. A method for detecting eEF-2 kinase and assessing eEF-2 kinase levels by:  
2 A. contacting a biological sample from a mammal in which the  
3 presence or activity of said eEF-2 kinase is suspected with a binding partner of said  
4 eEF-2 kinase under conditions that allow binding of said eEF-2 kinase to said  
5 binding partner to occur; and,  
6 B. detecting whether binding has occurred, and to what degree,  
7 between said eEF-2 kinase from said sample and the binding partner;

8 wherein the detection of binding indicates that presence or activity of said  
9 eEF-2 kinase in said sample, and indicates a level of said eEF-2 kinase in the  
10 sample.

1 27. An assay system for screening drugs and other agents for ability to modulate  
2 eEF-2 kinase activity, comprising a predetermined amount of eEF-2 kinase mixed  
3 with varying amounts of drug or agent, along with target protein and ATP; wherein  
4 detection is *via* either a detectable label on the  $\gamma$ -phosphate of ATP, or on an  
5 antibody directed against the phosphorylated target protein..

1 28. The assay system of Claim 27 wherein the label on the  $\gamma$ -phosphate of ATP  
2 comprises one of the following:

- 3 A.  $^{32}\text{P}$ ;
- 4 B.  $^{33}\text{P}$
- 5 C.  $^{35}\text{S}$
- 6 D. a biotinylated phosphate moiety; or,
- 7 E. a fluorescent phosphate moiety.

1 29. The assay system of Claim 27 wherein the label on the antibody comprises  
2 one of the following:

- 3 A. an enzyme detectable with colorimetric, fluorescent, or  
4 chemiluminescent substrates, such as alkaline phosphatase or horseradish  
5 peroxidase;
- 6 B. a biotin moiety;
- 7 C. a fluorescent moiety; or,
- 8 D. a radioactive moiety chosen from the following group of  
9 isotopes:  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{Co}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{90}\text{Y}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$ .

1 30. An assay system for screening drugs and other agents for ability to modulate  
2 eEF-2 kinase activity, comprising:

- 3                   A.     culturing an observable cellular test colony inoculated with a  
4 drug or agent;  
5                   B.     harvesting a supernatant from said cellular test colony; and,  
6                   C.     examining said supernatant for the presence of said eEF-2  
7 kinase activity wherein an increase or a decrease in a level of said eEF-2 kinase  
8 activity indicates the ability of a drug to modulate the activity of said eEF-2 kinase.

1 31.    A test kit for assessing the level of eEF-2 kinase activity in a eukaryotic  
2 cellular sample, comprising:

- 3                   A.    a predetermined amount of a detectably labelled specific binding  
4 partner of eEF-2 kinase.  
5                   B.    other reagents; and,  
6                   C.    directions for use of said kit.

1 31.    The test kit of Claim 31 wherein said labeled immunochemically reactive  
2 component is selected from the group consisting of polyclonal antibodies to eEF-2  
3 kinase, monoclonal antibodies to eEF-2 kinase, fragments thereof, and mixtures  
4 thereof.

1 32.    A method of preventing and/or treating cellular debilitations, derangements  
2 and/or dysfunctions and/or other disease states in mammals, comprising  
3 administering to a mammal a therapeutically effective amount of a material selected  
4 from the following group:

- 5                   A.    peptides that inhibit eEF-2 kinase;  
6                   B.    antibodies against eEF-2 kinase; and,  
7                   C.    other drugs or agents that specifically inhibit eEF-2 kinase.

1 33.    A pharmaceutical composition for the treatment of cellular debilitation,  
2 derangement and/or dysfunction in mammals, comprising:

3           A. a therapeutically effective amount of a material selected from the  
4 group consisting of: peptides that inhibit eEF-2 kinase; antibodies against eEF-2  
5 kinase; and, other drugs or agents that specifically inhibit eEF-2 kinase; and,

6           B. a pharmaceutically acceptable carrier.

1   34.    A recombinant virus transformed with the DNA molecule, or a derivative or  
2 fragment thereof, in accordance with Claim 18.

1   35.    A recombinant virus transformed with the DNA molecule, or a derivative or  
2 fragment thereof, in accordance with Claim 19.

1   36.    The recombinant DNA molecule of Claim 20 comprising plasmid pGEX-3X,  
2 clone E3 or plasmid pGEX-3X, clone E4.

1   37.    An antisense nucleic acid against eEF-2 kinase mRNA comprising a nucleic  
2 acid sequence hybridizing to said mRNA.

1   38.    The antisense nucleic acid of Claim 37 which is RNA.

1   39.    The antisense nucleic acid of Claim 37 which is DNA.

1   40.    The antisense nucleic acid of Claim 37 which binds to the initiation codon of  
2 any of said mRNAs.

1   41.    A recombinant DNA molecule having a DNA sequence which, on  
2 transcription, produces an antisense ribonucleic acid against eEF-2 kinase mRNA,  
3 said antisense ribonucleic acid comprising an nucleic acid sequence capable of  
4 hybridizing to said mRNA.

1 42. A eEF-2 kinase-producing cell line transfected with the recombinant DNA  
2 molecule of Claim 41.

1 43. A method for creating a cell line which exhibits reduced expression of eEF-  
2 kinase, comprising transfecting a eEF-2 kinase-producing cell line with a  
3 recombinant DNA molecule of claim 41.

1 44. A ribozyme that cleaves eEF-2 kinase mRNA.

1 45. The ribozyme of Claim 44 which is a *Tetrahymena*-type ribozyme.

1 46. The ribozyme of Claim 44 which is a Hammerhead-type ribozyme.

1 47. A recombinant DNA molecule having a DNA sequence which, upon  
2 transcription, produces the ribozyme of claim 44.

1 48. A eEF-2 kinase-producing cell line transfected with the recombinant DNA  
2 molecule of claim 47.

1 49. A method for creating a cell line which exhibits reduced expression of eEF-2  
2 kinase, comprising transfecting a eEF-2 kinase-producing cell line with the  
3 recombinant DNA molecule of claim 44.